and MCL5 (metabolically competent that express CYP1A1, 1A2, 2E1, 2A6, 3A4 and epoxide hydrolase) cells were determined after the cells (2.0×10^5) were treated for 24 h with the glucosinolate hydrolysis products; prop-2-enylisothiocyanate, 3-butenylnitrile, 3,4-epithiobutylnitrile, but-3-enylisothiocyanate, 4,5-epithiopentylnitrile and pent-4-enylnitrile. The glucosinolate hydrolysis products were synthesized as previously described (Luethy et al., 1981). Oxidative stress was determined fluorometrically using carboxy-H2DCFDA as an indicator of reactive oxygen species (ROS), as described by the manufacturer (Molecular Probes, USA).

A significant increase in oxidative stress for both prop-2-enylisothiocyanate (AITC) and 3,4 epithiobutylnitriles (3,4 ETN) in MCL-5 cells (Fig. 1B) was noted. No significant induction of ROS was found for 3-butenylnitrile (BN), but-3-enylisothiocyanate (BITC), 4,5-epithiopentylnitrile (4,5 ETN) and pent-4-enylnitrile (PN). In contrast, none of the six glucosinolate hydrolysis products induced ROS in the metabolically non-competent cHo1 cells, suggesting that P450s mediated oxidation was involved in ROS production by AITC and 3,4 ETN. The oxidative stress induced by prop-2-enylisothiocyanate has been associated with the electrophilicity of SH group due to a shorter methylene chain resulting in greater toxicity (Murata et al., 2000). However, the mechanism of ROS production by 3,4 epithiobutylnitrile is not understood. Both AITC and 3,4 ETN are cytotoxic and their ability to generate ROS mediated via cyp metabolism is likely to contribute to this toxicity. The lack of ROS induction by BN, BITC, 4,5 ETN and BN is consistent with their lack of toxicity in these cell lines.

References

IARC, 2004. IARC Handbooks of cancer prevention: cruciferous vegetables. Isothiocyanates and Indoles 9, 21–22.
Luethy, J., Von Daeniken, A., Friederich, U., Manthey, B., Zweifel, U., Schlatter, C., Benn, M.H., 1981. Synthesis and toxicological properties of three naturally occurring cyanoepithioalkanes. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene 72, 131–133.
Murata, M., Yamashita, N., Inoue, S., Kawanishi, S., 2000. Mechanism of oxidative DNA damage induced by carcinogenic allyl isothiocyanate. Free Radical Biology & Medicine 28 (5), 797–805.

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Release of soluble metal ions from copper based dental alloys measured by ICPMS

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Copper-based alloys have been used as an alternative to gold based alloys for dental crown and bridge applications. Their popularity is mainly due to their bright gold-like appearance and a lower cost when compared to alloys made from noble metals (Ardlin et al., 2009). Release of metal ions (Cu and Ni) and microparticles which may induce inflammation of the adjacent periodontal tissues and the oral mucosa has been documented in both *in vitro* and *in vivo* studies involving metallic dental devices (Geurtsen, 2002). The primary aim of this research project is to elucidate the release of metal ions from metallic dental alloys in a simulated oral environment.

Discs of copper-based dental alloy (NPG^{TM}+2) with the following nominal composition [in wt.%] copper 77.3:aluminium 7.8:nickel 4.3:iron 3.0:zinc 2.7:gold 2.0 and manganese 1.7 and NPG^{TM} alloy (without gold) were obtained from Dentech Dental, London, UK. Specimens of NPG^{TM}2 and NPG^{TM} alloys (32 mm × 10 mm × 1.5 mm) were totally immersed in 10 mL of sterile artificial saliva (Mariano et al., 2009) at both neutral (pH 7.0) and acidic pH (pH 4.0) (acidic milieu simulating the condition of plaque build-up in inter-proximal space between adjacent teeth). The tubes were sealed and incubated at 37 °C for 7 days with agitation. Saliva extracts were analysed for metal ion concentrations (Cu, Ni, Zn, Al and Mn) using ICP-MS.

Metal ion release from both alloys in artificial saliva at pH 4.0 was significantly higher (for all the metals, and at all incubation times) when compared to the levels observed at pH 7.0 (2 sample *t*-test $p \leq 0.05$). Table 1 shows the extent of corrosion observed at pH 7.0 and 4.0 for the NPG^{TM} alloy, at incubation times 1, 3, 5 and 6 days. As would be expected from the composition, Cu ions are released to the greatest extent on corrosion. The greatest increase in corrosion, comparing pH 7.0 and 4.0, was measured with Mn (90 fold increase), followed by Ni (74 fold increase) and Zn (30 fold increase). No significant difference in ion release was observed in the extent of corrosion between the two alloys at either pH 7.0 or 4.0 (2 sample *t*-test $p \geq 0.05$).

The results show that corrosion of the Cu alloy with resultant release of metal ions in artificial saliva was most notable at acidic pH (pH 4.0) which simulates the condition of plaque build-up in inter-proximal space between adjacent teeth. NPG^{TM}2 was not superior to NPG^{TM} in terms of resistance to metal ion release despite its Au content. The 2% Au content in NPG^{TM}2 is probably not high enough to make a significant difference in ion release between the two alloys. The marked release of Cu and Ni is potentially a significant local and systemic toxicity problem in patients with dental implants, Ni being particularly associated with marked allergenicity.

References

Ardlin, B.I., Lindholm-Sethson, B., Dahl, J.E., 2009. J. Biomed. Mater. Res. B Appl. Biomater. 88, 465–473.
Geurtsen, W., 2002. Crit. Rev. Oral Biol. Med. 13, 71–84.
Mariano, N.A., Oliveira, R.G., Fernandes, M.A., Rigo ECS, 2009. Matéria (Rio J.) 14 (2), 878–880.

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Table 1
Metal ion release [concentration (mean ± SEM; μg/L, $n = 3$)] from NPG^{TM} alloy at pH 7.0 and 4.0 at incubation times 1, 3, 5 and 6 days.

Time	pH	Al	Mn	Ni	Cu	Zn
Day1	7.0	37.69 ± 5.24	16.92 ± 4.36	17.76 ± 5.08	826.01 ± 118.50	31.14 ± 8.33
Day1	4.0	98.74 ± 24.09	544.6 ± 45.91	612.95 ± 106.13	2736.21 ± 107.11	672.74 ± 129.55
Day3	7.0	33.01 ± 10.55	24.14 ± 7.72	16.60 ± 4.37	805.22 ± 177.92	38.99 ± 7.01
Day3	4.0	88.59 ± 11.40	542.23 ± 35.19	641.38 ± 59.47	3305.82 ± 992.04	613.03 ± 37.23
Day5	7.0	27.97 ± 19.34	24.86 ± 3.90	16.49 ± 1.66	790.15 ± 199.53	24.97 ± 11.84
Day5	4.0	410.64 ± 298.18	1029.81 ± 301.16	1188.33 ± 359.27	3281.9 ± 931.40	772.96 ± 26.45
Day6	7.0	21.50 ± 9.57	16.21 ± 6.32	13.97 ± 3.83	752.93 ± 184.29	24.80 ± 11.94
Day6	4.0	195.65 ± 77.62	1413.15 ± 816.25	740.14 ± 138.10	2133.16 ± 565.69	748.94 ± 61.07